REMARKS

Claims 1-20 and 58 are pending and under examination. New claims 59-79 have been added. Support for the new claims can be found throughout the specification and the claims as filed. In particular, support for new claims 59-79 can be found in original claims 1-20 and, for example, on page 55, lines 15-17, and page 58, line 25, to page 59, line 11. Accordingly, these new claims do not raise an issue of new matter and entry thereof is respectfully requested. Applicants point out that new claims 59-79 are directed to *in vitro* methods.

The rejection of claims 1-20 and 58 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods.

The claimed methods are directed to methods of differentiating progenitor cells by contacting progenitor cells with a differentiating agent and introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing protected neuronal cells. Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance to enable one skilled in the art to make and use the methods as claimed. Applicants respectfully disagree with the assertion on page 3 of the Office Action that the specification provides only general guidance for treatment of neurodegenerative disease or little to no guidance on *in vivo* uses of the method. The specification teaches that the methods can be performed *in vitro*, and the differentiated progenitor cells generated by the method can be transplanted into a patient having, for example, a neurodegenerative disease (page 57, line 14, to page 58, line 24). The specification also teaches that the differentiated progenitor cells can be transplanted into the brain, eye (retina) or spinal cord after neuronal injury or damage using well known methods (page 57, lines 19-21, and page 58, lines 17-24). The specification additionally teaches that cells can be transplanted into a patient prior to, during or after differentiation of the progenitor cells into neuronal cells and that the neuronal environment can drive the cells into the desired neuronal cell type due to the presence of the appropriate environmental cues (page 58, line 29, to page 59, line 11). The specification further teaches various methods for introducing a nucleic acid molecule into a progenitor cell, including well known methods for introducing a nucleic acid *in*

vivo (page 54, line 4, to page 55, line 17). Accordingly, Applicants respectfully maintain that the specification provides sufficient description and guidance for treating a neurodegenerative disease and for *in vivo* uses of the claimed methods.

In the Office Action, various references are discussed as supporting the alleged unpredictability of the claimed methods, and it is asserted that successful implementation of cell therapy and gene therapy protocols was not routinely achievable by those skilled in the art. Regarding Rossi and Cattaneo, Nat. Rev. Neurosci. 3:401-409 (2002), the Office Action refers to the abstract as indicating that "stem cell therapy for neurological disorders is still a distant goal" and the need for homogeneous populations of neural stem cells and the need to understand the mechanisms for "their [neural stem cells] proper integration into the injured brain." The Office Action further refers to functional integration of donor cells as remaining a highly demanding task, referring to page 401, column 2, paragraph 2, last sentence. This passage reads as follows:

> The recent breakthroughs in stem cell research have opened up new possibilities for cell-replacement therapy to treat neurological diseases. Given their expected capacity to self-renew and differentiate into the desired cell type, clonal populations of neural stem cells promise to produce similar if not more beneficial effects, while being more amenable to manipulation, enrichment and expansion. Nonetheless, the functional integration of donor cells remains a highly demanding task that requires a profound understanding and control of the biological properties of both donor cells and the host environment. [column 2, paragraph 2, cited references omitted]

Thus, the "donor cells" clearly refer to "neural stem cells." However, the claimed methods are directed to differentiating progenitor cells to produce a cell population containing protected neuronal cells. This is achieved by contacting the progenitor cells with a differentiating agent and introducing into the progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof. As taught in the specification, a stem cell refers to a "pluripotent cell type which can differentiate under the appropriate conditions to give rise to all cellular lineages" (page 29, lines 7-10). This is in contrast to the differentiated progenitor cells generated by the claimed method. Accordingly, the issues that may exist for generating homogeneous populations of neural stem cells or the functional integration of donor neural stem cells and their biological properties, as discussed in Rossi and Cattaneo, are not relevant to the claimed methods producing protected neuronal cells.

Referring to Cao et al., J. Neurosci. Res. 68:501-510 (2002), this reference, like Rossi and Cattaneo, also discusses issues relating to "neural stem cells." The Office Action indicates that Cao et al. acknowledges "the potential use of stem cells in therapeutic transplantation and for *in vivo* manipulation of endogenous precursors." However, Applicants respectfully point out that Cao et al. discusses the use of neural stem cells for repair of central nervous system injury, not the use of stem cells in general (see abstract). Cao et al. indicates that neural stem cells can be isolated from embryonic or adult brain tissue or from embryonic stem cells (page 501, right column). Therefore, Cao et al. clearly describes the use of neural stem cells, not differentiated cells as generated in the claimed methods.

In the Office Action, it is asserted that Cao et al. emphasizes that "this at present is challenging and so far has been unsuccessful," referring to the abstract and page 507, column 2, paragraph 2. These passages read as follows:

> Manipulation of endogenous neural precursors may be an alternative therapy or a complimentary therapy to stem cell transplantation for neurodegenerative disease and CNS injury. However, this at present is challenging and so far has been unsuccessful. Understanding mechanisms of NSC differentiation in the context of the injured CNS will be critical to achieving these therapeutic strategies. [abstract]
>
> There is great potential for the ultimate manipulation and directed differentiation of endogenous stem cell populations, but this is at present a challenging and as yet unsuccessful approach. [page 507, second column, second paragraph]

Thus, Cao et al. describe the manipulation of endogenous neural precursors as challenging and unsuccessful. The Office Action further indicates that Cao et al. points out that understanding the "mechanisms of NSC differentiation in the context of injured CNS will be critical to achieving these therapeutic strategies," referring to the abstract and page 507, column 2, paragraph 2. However, again, Cao et al. is referring to mechanisms of neural stem cell differentiation of endogenous neural stem cells. In contrast, the claimed methods are directed to differentiating progenitor cells by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide or active fragment thereof. Therefore, the issues with respect to the manipulation of endogenous

neural precursors in general are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*.

Regarding Milward et al., J. Neurosci. Res. 50:862-871 (1997), the Office Action asserts that this reference "demonstrates that transplantation of neural stem cells (NSCs) to the CNS does not produce a therapeutic effect in a diseased animal." The Office Action acknowledges that the reference describes the transplantation of canine neural stem cells into both a myelin-deficient rat model and a shaking pup myelin mutant dog. The Office Action further acknowledges that Milward et al. disclosed that the graft-derived cells produced myelin in the rat model and that the grafted cells integrated normally into the adult shaking pup cytoarchitecture. The Office Action then states the following:

> Yet despite all this, the clinical deficit of these animals was not ameliorated. Thus, it is clear that the production of myelin *in vivo* and normal integration of cells is not predictive of a therapeutic outcome. [page 5, first paragraph]

However, upon review of Milward et al., this reference is completely silent as to any therapeutic effect of the transplanted cells in either the rat or dog model and makes no statement or implication whatsoever that production of myelin *in vivo* is not predictive of a therapeutic outcome. Milward et al. states:

> Transplantation of lacZ-expressing *wt* neurospheres into the myelin-deficient (*md*) rat showed that a proportion of the cells differentiated into oligodendrocytes and produced myelin. In addition, cells from the neurosphere populations survived at least 6 weeks after grafting into a 14-day postnatal *sh* pup recipient and at least 2 weeks after grafting into an adult *sh* pup recipient. Thus, neurospheres provide a new source of allogeneic donor cells for transplantation studies in this mutant. [abstract, last three sentences]
>
> Moreover, grafting of w*t* neurospheres into *md* recipient spinal cords resulted in the production of apparently normal myelin by graft-derived cells, showing that these cells are able to myelinate a myelin-deficient host. [page 868, second column, first paragraph]

The silence in Milward et al. as to whether there was a therapeutic benefit in no way supports the assertion in the Office Action that "the clinical deficit of these animals was not

ameliorated." Applicants respectfully request that the Examiner provide the basis for such an assertion or withdraw the assertion from the record.

With respect to Mehler et al., Arch. Neurol. 56:780-784 (1999), the Office Action asserts that this reference discloses that many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitors to realize their broad lineage potential. It is further asserted that specific neuropathological conditions may alter the normal balance of regional environmental signals and that inappropriate cellular cues may predispose residual neural populations to undergo apoptosis. The Office Action quotes Mehler et al. as indicating that "[t]his suggests that it may be necessary to promote lineage commitment of progenitor cells *in vitro* prior to transplantation into a damaged brain," referring to page 782, column 1, paragraph 1.

As discussed above, the claimed methods are directed to differentiating progenitor cells by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide or active fragment thereof. Therefore, the issues with respect to environmental cues for differentiation of neural progenitor cells that may or may not be present in normal or neuropathological conditions are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*. Moreover, as discussed above, Milward et al., *supra*, describes appropriate differentiation and production of myelin from neurospheres transplanted *in vivo* in a myelin-deficient rat model, indicating that appropriate differentiation can occur in a neuropathological condition (also see discussion below).

The Office Action asserts that the specification fails to provide an enabling disclosure for the genetic modification of human ES cells. With regard to genetic modification of human ES cells, Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance to enable the use of human ES cells. As discussed previously, for example in the response filed September 3, 3004, the specification teaches that well known methods for introducing a nucleic acid molecule into progenitor cells such as embryonic stem cells include microinjection, electroporation, and lipofection as well as viral-mediated techniques such as retroviral,

lentiviral and adenoviral transduction (page 54, line 4, to page 55, line 17). Thus, the specification clearly teaches a variety of techniques that can be used to introduce a nucleic acid molecule into progenitor cells.

Furthermore, Applicants previously provided in the response filed September 3, 2004, corroborative evidence that one skilled in the art would have been able to introduce a nucleic acid molecule into human progenitor cells. In particular, Cheng et al., Blood 92:83-92 (1998), described an optimized retroviral gene-transfer protocol clinically applicable to gene transfer into human hematopoietic stem cells (submitted as Exhibit A in response filed September 3, 2004). In the Office Action, it is asserted that, contrary to "Applicants' suggestion," the retroviral gene transfer protocol described by Cheng et al. was limited to *in vitro* uses and that Cheng et al. does not teach protocols for using MSCV to transfect human hematopoietic stem cells *in vivo*. Applicants respectfully point out that there was no explicit "suggestion" in Applicants' remarks that Cheng et al. taught protocols for using MSCV to transfect human hematopoietic stem cells *in vivo*, rather, it was stated that Cheng et al. described "an optimized gene-transfer protocol clinically applicable to gene transfer into human hematopoietic cells" (responses filed September 3, 2004, and May 31, 2005, emphasis added). Applicants respectfully maintain that this is an accurate characterization of the teaching of Cheng et al. In particular, Cheng et al. states:

> Based on the outcome of this study, it would appear that MSCV-based vectors may offer advantages over conventional MoMLV-based vectors for gene delivery to the human hematopoietic system. The ability to direct sustained high-level expression of exogenous genes in differentiated cells derived from transduced HSC/progenitors is obviously a desirable goal of a number of human gene therapy protocols targeting congenital blood disorders. [page 91, first complete paragraph, emphasis added, cited reference omitted]

Furthermore, Hanazono et al., Stem Cells 19:12-23 (2001), which is referred to in the Office Action as acknowledging that Cheng et al. demonstrates the use of murine stem cell virus, describes murine stem cell virus vectors as having been "shown to result in improved expression of transgenes compared to MoMLV vectors *in vivo* in mice, and *in vitro* in human primary hematopoietic cells" and that "[t]hese vectors are being examined *in vivo* in

nonhuman primate models" (quoted in Office Action and referring to page 16, column 2, paragraph 1 of Hanazono et al.). Thus, Hanazono et al. discusses the well known use of viral vectors for *in vivo* gene therapy applications. Applicants respectfully submit that the optimized retroviral gene-transfer protocol described by Cheng et al. is relevant to *in vivo* uses since such viral vectors are routinely used for *in vivo* transduction, as acknowledged in Hanazono et al., and, therefore, the teaching of Cheng et al. is relevant to the scope of the claimed methods.

Zwaka and Thomson, Nat. Biotechnol. 21:319-321 (2003), was previously submitted to corroborate Applicants' position that electroporation can be used to produce stably transfected human ES cells (submitted as Exhibit C with response filed September 3, 2004). In the Office Action, Zwaka and Thomas is asserted to be a post-filing reference that used a unique electroporation protocol that was specially adapted for human ES cells. While it is acknowledged that Zwaka and Thomson is a post-filing reference that describes an improved electroporation method for human ES cells, Applicants respectfully submit that Zwaka and Thomson nevertheless teaches the use of known electroporation techniques that were successful for transfecting human ES cells, albeit at lower efficiencies than their improved technique. First, Zwaka and Thomson describe the development of an improved electroporation protocol for human ES cells not because human ES cells could not be transfected by electroporation but because the lower transfection efficiency did not allow efficient homologous recombination of the transfectants, the goal of this particular study. As indicated in the Office Action, Zwaka and Thomson do indicate that there are significant differences between mouse and human ES cells, but this is stated in the context that such differences "have hampered the development of homologous recombination in human ES cells" (see abstract). Zwaka and Thomson state:

> Our failure to achieve homologous recombination with chemical transfection reagents led us to re-evaluate electroporation procedures for human ES cells. In our hands, electroporation using a typical mouse ES cell protocol (220 V, 960 μF, electroporation in PBS) yielded a stable transfection rate of ~10^{-7}. Given current culture techniques, this frequency is too low to be practical for identifying rare homologous recombination events. [page 319, second column, first complete paragraph, emphasis added, cited reference omitted]

Thus, Zwaka and Thomson in fact teach that a standard electroporation protocol does result in stably transfected human ES cells, albeit at a frequency too low to be practical for their described goal of homologous recombination. Therefore, Applicants respectfully maintain that Zwaka and Thomson corroborates Applicants position that electroporation can be used to produce stably transfected human ES cells.

Eiges et al., Curr. Biol. 11:514-518 (2001), is an additional reference provided to corroborate Applicants' position that electroporation can be used to transfect human ES cells (submitted as Exhibit B in response filed September 3, 2004). The Office Action acknowledges that Eiges et al. compared the efficiency of several different transfection protocols for human ES cells. Human ES cells were successfully transfected using electroporation and two commercially available transfection reagents, Fugene and ExGen 500, with ExGen 500 showing the highest efficiency (see Figure 1). The Office Action asserts that Eiges et al. would not have been available to the skilled artisan since it is a post-filing reference. However, Applicants respectfully maintain that Eiges et al. corroborates Applicants' position that routine transfection methods, including electroporation, could be used to successfully transfect human ES cells, as demonstrated in Figure 1.

Furthermore, Eiges et al. teaches that other routine transfection methods such as Fugene and ExGen 500 could also be used to successfully transfect human ES cells. To corroborate Applicants position that transfection methods such as Fugene and ExGene 500 were routine and available to one skilled in the art at the time of filing of the priority application, June 5, 2000, submitted herewith is a reference by Ferrari et al., Gene Therapy 4:1100-1106 (1997)(Exhibit A), describing ExGen 500 as an efficient transfection reagent. Also submitted is a reference by Uyttersprot et al., Mol. Cell. Endocrin. 142:35-39 (1998)(Exhibit B), describing the use of commercially available FuGENE™ 6 (purchased from Boehringer Mannheim, see page 36 under "Transfection procedure"). Thus, Eiges et al. describes the successful transfection of human ES cells using routine methods well known to those skilled in the art at the time of filing of the priority application. Applicants respectfully submit that it would have been routine for one skilled in the art to try various known transfection methods to successfully transfect human ES cells, as corroborated by Eiges et al.

The Office Action on page 9 discusses Applicants' previous arguments regarding transient expression, antibiotic selection and transfection efficiency. For example, antibiotic selection is indicated to be an *in vitro* technique not applicable to *in vivo* transfection. The Office Action discusses Applicants' previous arguments that the claimed methods of differentiating progenitor cells do not require a particularly high efficiency of transfection of human or other progenitor cells. Applicants respectfully point out that the context of Applicants' discussion about transfection efficiency and the use of antibiotic selection was based on the assertion in the Office Action mailed November 28, 2003, that human stem cells are more difficult to transfect than murine stem cells, particularly in view of Eiges et al. Clearly the techniques described by Eiges et al. are *in vitro* techniques, and Applicants respectfully submit that such arguments are relevant to the issue being asserted in the Office Action regarding the efficiency of *in vitro* techniques. Applicants respectfully maintain, as discussed in the response filed March 29, 2004, that the claimed methods of differentiating progenitor cells do not recite, nor do they require, a particularly "high efficiency" of transfection. Rather, one skilled in the art understands that cell populations stably expressing an introduced nucleic acid molecule can be routinely prepared using, for example, standard methods such as antibiotic selection in order to select for a transfected population of cells. Thus, the skilled person understands that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able produce a population of progenitor cells predominantly or uniformly containing a MEF2 polypeptide using only routine methods.

The Office Action acknowledges Applicants' arguments regarding the description in Jackowski, Br. J. Neurosurg. 9:303-317 (1995), of the presence *in vivo* of neurotrophic activities in the diseased or injured tissue environment. However, the Office Action asserts that the description in Jackowski of peripheral nerve transection resulting in the production of large quantities of NGF being produced is an injury, not "an ongoing pathological process" as with the therapeutic applications of the claimed methods. Similarly, McDonald et al., Nat. Med. 5:1410-1412 (1999)(submitted as Exhibit E in response filed September 3, 2004), and Liu et al., Proc. Natl. Acad. Sci. USA 97:6126-6131 (2000)(submitted as Exhibit F in response filed September 3, 2004), both of which were provided submitted to corroborate Applicants' position that differentiation can occur *in vivo* in animal models of

nerve injury or disease, are asserted in the Office Action to relate to injury, not an ongoing pathological process.

Applicants respectfully maintain that differentiation can occur *in vivo* in injury and disease. For example, as discussed above, Milward et al. describes differentiation of transplanted neurospheres into oligodendrocytes in a myelin-deficient rat model, which is an ongoing pathological process, not an injury model (see abstract). Milward et al. further states:

> Myelin deficiency may activate compensatory mechanisms. Even when these are insufficient to override effects of endogenous myelin gene mutations, the environment may nonetheless express factors favoring oligodendrogenesis and myelinogenesis by transplanted cells. [page 869, first column, last paragraph]

Thus, Milward et al. corroborates that differentiation can occur *in vivo* in a disease model.

Furthermore, Rossi and Cattaneo, *supra*, describes *in vivo* differentiation of fetal cells in experimental animal models and human patients with Huntington's disease.

> In the case of Huntington's disease, in which a mutant gene causes the selective death of striatal neurons, functional recovery requires at least partial reconstruction of a complex cortico-striato-pallidal circuit. However, the selective cell death, and the vicinity and accessibility of host pallidal targets for donor axons originating in the striatum, together with the genetic nature and slow progression of the disease, make it a good candidate for cell transplantation. Accordingly, in both experimental animals and human patients, fetal cells that have been grafted into the striatum are correctly innervated by host neurons and send their axons to the adjacent globus pallidus. Important behavioral and cognitive improvements have been observed in patients, which are thought to be related to the extent of reconstruction of the striatal circuitry. Furthermore, in the studies completed so far, and at the times that have been considered after transplantation, implanted cells do not seem to be affected by the underlying genetic dysfunction of Huntingtons's disease. [page 402, paragraph bridging columns 1 and 2, cited references omitted).

Accordingly, Applicants respectfully submit that Rossi and Cattaneo corroborate Applicants' position that differentiation can occur in disease and injury.

Further in corroboration of the enablement of the claimed methods, submitted herewith as Exhibit C (with Exhibits 1 through 5 attached thereto), is a Rule 132 Declaration by Dr. Lipton, one of the inventors of the present application. Exhibit C describes experimental results showing that transplanted MEF2CA neural stem cells survive, migrate and differentiate into neurons in the ischemic mouse cerebral cortex. Applicants respectfully submit that the evidence provided in Exhibit C corroborates the enablement of the claimed methods.

For the reasons of record and as discussed above, and further in view of the corroborative evidence submitted herewith, Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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